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Inhibins and activins regulate mammary epithelial cell differentiation through mesenchymal-epithelial interactions

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SUMMARY

Inhibins and activins are members of the transforming growth factor beta (TGFβ) family. Female mice in which both alleles encoding the inhibin βB subunit have been deleted are unable to nurse their pups. We have now identified a cause of lactation failure in these mice. Ductal elongation and alveolar morphogenesis are retarded. During puberty and pregnancy, ductal outgrowth and alveolar development are limited and morphologically abnormal endbuds persist in the glands of postpartum females. The alveolar lumina fail to expand at parturition due to the absence of secreted milk. Transplantation experiments have been performed to determine whether the absence of systemic- or mammary-derived βB subunits are the cause for the incomplete and aberrant development. While transplanted intact glands from wild-type mice grew normally in βB-deficient hosts, βB-deficient glands remained underdeveloped in wild-type hosts. However, βB-deficient epithelium developed normally when transplanted into the fat pad of wild-type hosts. This demonstrates that ductal elongation and epithelial cell differentiation during puberty and pregnancy require activin/inhibin signalling from the stroma. The results further show that distinct, though related, activins and inhibins perform unique functions and are not able to compensate for the absence of activin B and AB and inhibin B in the process of mammogenesis. The βB-deficient mice provide the first genetic evidence for stromal signalling in the adult mammary gland in vivo.

Key words: mammary development, ductal growth, lactogenesis, mammary stroma, mouse, transforming growth factor, βB

INTRODUCTION

Activins and inhibins are members of the TGFβ superfamily, a class of dimeric glycoproteins that display a wide spectrum of activities (for a review see Vale et al., 1994). Activins function as dimers of two β-subunits, βA and βB. Three types of activins have been isolated: activin A (βAβA), activin B (βBβB) and activin AB (βAβB). Inhibins share a common α subunit associated with βA (inhibin A) or βB (inhibin B). Activins and inhibins were first identified as gonadal factors that influence the production of follicle-stimulating hormone in the pituitary. Subsequently, they have been shown to play complex roles in neuroendocrine regulation and also modulate luteotropic hormone, growth hormone and adrenocorticotropic hormone production. In addition, they affect gonadal functions such as steroid production and regulate placental hormone synthesis. In these processes, activins and inhibins frequently have opposing effects and in in vitro assays the three activins and the two inhibins display interchangeable activities (Vale et al., 1994). Several other functions in development have been deduced from in vitro assays. For example, activin A is able to induce mesoderm formation in *Xenopus* embryos in a concentration-dependent manner. It also induces proliferation and terminal differentiation of erythroid precursor cells and stimulates bone formation. In the central nervous system, activin seems to promote oxytocin release from the neurohypophysis. Consistent with the manifold functions, activins and inhibins show a broad tissue distribution (Vale et al., 1994).

Analyses of their function in vivo have been initiated recently through the inactivation of individual subunits and receptors by homologous recombination in mice (Matzuk, 1995). Inactivation of the α subunit results in the absence of inhibins and causes infertility due to the development of gonadal tumors at an early age (Matzuk et al., 1992). Independent inactivation of the *βA* and *βB* genes has demonstrated that the βA and βB subunits display non-overlapping activities. In βB-deficient mice, the activities of activins B and AB as well as inhibin B are eliminated (Vassalli et al., 1994). βB-deficient pups are born with open eyelids but their overall development is normal. Fertility of the females is slightly reduced and a prolonged gestation time may be indicative of systemic endocrine defects. Most prominently, females exhibit a lactational defect and cannot support their litters. The inactivation of the *βA* gene eliminates activin A and AB as well as inhibin A and causes malformation of the secondary palates and an absence of teeth and whiskers (Matzuk et al., 1995b). Since the newborn mice are incapable of suckling and die within 24 hours, the effects on mammary gland development are not known. Mice deficient in both βA and βB exhibit a combination of the phenotypes seen in each of the mutants but have no additional defects (Matzuk et al., 1995b).

The development of the mammary gland proceeds in distinct phases and functional differentiation of secretory epithelial cells

is a critical step in the reproductive cycle of mammals. Pronounced ductal growth occurs at the onset of puberty and extensive development in cycling virgins leads to the formation of a ductal tree, which fills the entire mammary fat pad (Daniel and Silberstein, 1987). Alveolar proliferation occurs during pregnancy and terminal differentiation of alveolar epithelial cells is completed at the end of gestation with the onset of milk secretion at parturition (Topper and Freeman, 1980). In order to understand the roles of inhibins and activins in mammary gland function, we studied mammogenesis in βB-deficient mice. In particular, we determined whether systemic- or mammary-derived activins and inhibins are required for mammary development.

MATERIALS AND METHODS

Mice

The generation of βB-deficient mice by homologous recombination was described previously (Vassalli et al., 1994). Hemizygous βB mutant mice were kindly provided by Drs Vassalli and Jaenisch and mated to generate null mice. For dated pregnancies, the mice were mated and inspected daily for vaginal plugs. The day after copulation was counted as day 0 of pregnancy.

Whole mounts and histology

The first inguinal gland (no. 4) was removed at the indicated times of development and spread on a glass slide. After fixation for 2 to 4 hours in Carnoy's solution, the glands were hydrated and stained with carminalum, dehydrated and mounted as described before (Kordon et al., 1995). The glands were photographed and embedded in paraffin. 5 μm sections were prepared by standard methods, stained with hematoxylin and eosin and used for histological analysis.

RNA isolation and northern blots

Glands were homogenized in guanidinium thiocyanate and RNA was extracted according to Chomczynski and Sacchi (1987). 20 μg of total RNA was separated in 1.5% formaldehyde gels, blotted onto nylon membranes and probed with ^{32}P-labelled probes as described before (Robinson et al., 1996). The mACT10/11;13 probe was used to detect βB transcripts (Vassalli et al., 1994).

Transplantations

Two types of transplantations were performed. For fat pad transplantations, the entire fat pad was removed at 3-4 weeks of age and replaced with the fat pad of another mouse. For transplantations of mammary epithelium, the endogenous epithelium was removed as described by DeOme et al. (1959). An incision was made in the cleared fat pad and a small piece of the donor mammary gland was transplanted. Two months after surgery, the host mice were mated and the transplanted tissues were harvested during pregnancy or within 12 hours of delivery.

RESULTS

Epithelial development

After delivering regular-sized litters, βB-deficient females exhibited maternal behavior and attempted to nurse their young. The pups died within 24 hours after birth and no milk was detected in their stomachs. However, pups could be fostered onto wild-type and hemizygous dams suggesting that βB-deficient females failed to lactate. To identify the cause of lactation failure, we analyzed mammary whole mounts at different times during pregnancy and evaluated ductal and alveolar morphogenesis. The ductal tree in 4-week-old immature βB-deficient females (Fig. 1A) was indistinguishable from that in wild-type littermates (Fig. 1B). Striking differences were observed in mature virgins (Fig. 1C,D). While the mammary fat pad in 2-month-old wild-type females was completely filled with branched ducts (Fig. 1D), ducts in the inguinal fat pads of mutant females had not penetrated beyond

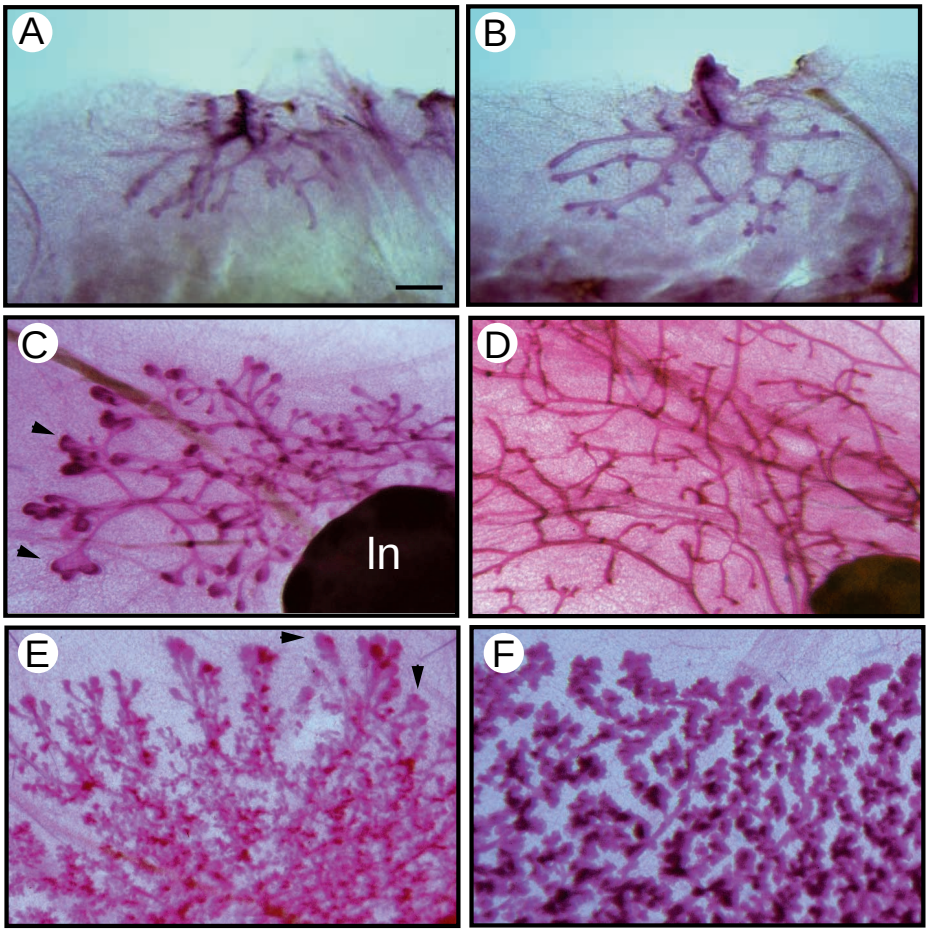

Fig. 1. Ductal growth in *βB*$^{-/-}$ mice. Mammary gland whole mounts from *βB*$^{-/-}$ (A,C,E) and control (B,D,F) mice. (A,B) Tissue from 4-week-old mice. The difference in size between the two glands is within the range of variability between different glands at this stage. C) *βB*$^{-/-}$ tissue at 3 months. The mammary ducts have grown to the vicinity of the lymph node (ln). Terminal end buds (arrowheads) indicate areas of active ductal elongation. The ductal tree proximal to the lymph node shows well-developed side branches. (D) Tissue from wild-type mouse at 2 months. The ductal tree has penetrated the entire fat pad and terminal end buds have disappeared. (E,F) Tissue at day 18 of pregnancy. (E) Incomplete filling of the fat pad is seen in *βB*$^{-/-}$ mice and terminal end buds are still persisting (arrowheads). (F) The fat pad of control mice is filled with secretory alveoli. Bar, 500 μm (A,B); 400 μm (C,D); 250 μm (E,F).

the lymph node (Fig. 1C). Profound differences were also visible in the endbuds, the sites of ductal elongation. While end buds had disappeared in 2-month-old wild-type females indicating the completion of ductal elongation (Fig. 1D), they persisted in 3-month-old βB-deficient females (Fig. 1C). Ductal outgrowth and alveolar development in βB-deficient mice (Fig. 1E) during pregnancy lagged behind that seen in control mice (Fig. 1F) and was not completed at the end of pregnancy. Although the proximal part of the gland was well developed, end buds were still visible in the distal region of the epithelial tree, which had not reached the end of the fat pad (Fig. 1E).

Alveolar development

Whole mounts shown in Fig. 1 were sectioned, and ductal and alveolar development was evaluated at the cellular level. Whereas limited ductal outgrowth and prominent endbuds were observed in 3-month-old βB-deficient mice (Fig. 2A,C,E), an elaborate ductal system lacking endbuds was apparent in 2-month-old control mice (Fig. 2B,D,F). Some end buds in βB-deficient mice exhibited a normal appearance with a cap cell layer (Fig. 2E), but the majority showed a disorganized pattern as described below.

Sections through mammary whole mounts from late pregnant (Fig. 3A,C,E) and postpartum (Fig. 3B,D,F) mice revealed an even more pronounced failure of ductal outgrowth and severe underdevelopment of lobulo-alveolar structures in βB-deficient mice. At late pregnancy (Fig. 3A), the alveoli were sparse and small, and they contained round cells with large nuclei. Very little secretion was found in the lumina (Fig. 3A). The persisting end buds in late pregnant and postpartum βB-deficient mice had a disorganized appearance. The cap cell layer was missing, mitoses were frequently seen in the body cells and the central lumen was often absent (Fig. 3C,D). Concomitant with the underdevelopment of the alveoli, the stroma contained many adipocytes (Fig. 3A-D) while they were mostly replaced by alveolar epithelial cells in the wild-type glands (Fig. 3E,F). After parturition, the alveoli of βB-deficient mice were still small, the secretory cells remained rounded and the small lumen contained a dense secretion (Fig. 3B). In contrast, alveoli of postpartum control mice displayed the typical flattened appearance of secretory cells and exhibited extended lumina (Fig. 3F).

Expression of the *βB* gene in mammary tissue

In order to determine whether βB is produced in the mammary gland and whether the absence of mammary-derived βB subunits could account for the aberrant mammary development, we analyzed the βB expression pattern in mammary tissue during pregnancy. An approximately 4.5 kb transcript corresponding to the major βB mRNA was detected in mammary tissue from virgin mice, throughout pregnancy and during lactation (Fig. 4A). The expression of the 3.5 kb transcript followed an identical pattern (Fig. 4A). Neither the 4.5 nor 3.5 kb transcript could be detected in mammary glands of βB-deficient mice (Fig. 4A). The 4 kb transcript in mammary tissue from mice carrying mutated βB alleles is likely to correspond to a neo-βB transcript (Vassalli et al., 1994). Expression of the *βB* gene in mammary tissue is constitutive throughout puberty, pregnancy and lactation.

Epithelial differentiation

Hallmarks of mammary epithelial cell differentiation are the transcriptional activation of milk protein genes, followed by the synthesis and secretion of the corresponding proteins. Although mammary tissue of βB-deficient mice did not fully develop and fill the fat pad during pregnancy, it was unclear whether the alveoli in these mice differentiated with parturition. We addressed this issue by analyzing the expression patterns of milk protein genes. Transcription of the genes encoding WAP and β-casein (Fig. 3B), and α-lactalbumin and WDNM1 (data not shown) in late pregnant and postpartum tissue of βB-deficient mice was similar to that seen in hemizygous or wild-type littermates (Fig. 4B). Although βB-deficient mice contain less mammary tissue, the alveolar

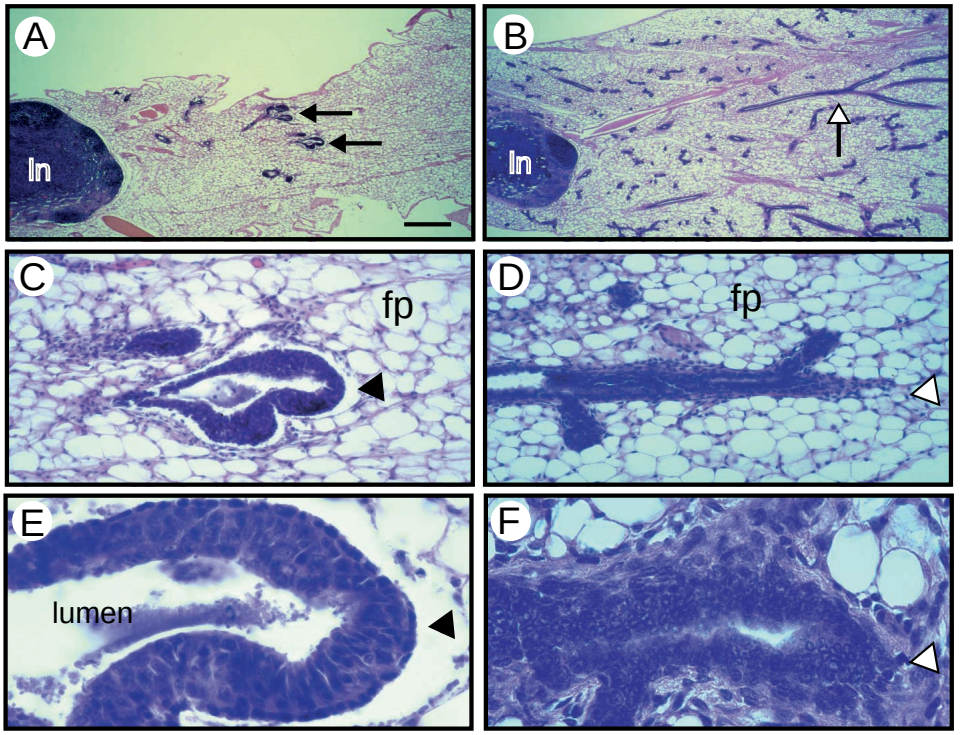

Fig. 2. Histological analysis of virgin glands. (A,C,E) Ducts and endbuds from 3-month-old $\beta B^{-/-}$ and (B,D,F) 2-month-old wild-type mice. The whole mounts shown in Fig. 1C and D were sectioned and stained with H&E. ln, lymph node; fp, fat pad. The solid arrows in A and the solid arrowheads in C and E point to endbuds. The white arrow in B points to a duct and the white arrowheads in D and F indicate the terminus of a duct. Note, no endbuds are visible in the control mice. Bar, 300 μm (A,B); 80 μm (C,D); 100 μm (E,F).

epithelial cells clearly exhibit a differentiation phenotype compatible with milk protein gene transcription. By immunohistochemistry, we saw WAP in the lumen of βB-deficient mice (data not shown).

Effect of mammary-derived versus systemic inhibin βB

The steady state level of βB mRNA in mammary tissue is higher than in most tissues and comparable to that seen in ovaries, suggesting that locally produced βB may modulate mammary development and functional differentiation through an autocrine, paracrine or intracrine mechanism. Alternatively, mammogenesis may be dependent on the systemic effects of βB. To distinguish between these possibilities, we performed a series of mammary transplantation experiments as outlined in Fig. 5.

Transplantation of the entire mammary fat pad containing the mammary anlage from a wild-type mouse into a βB-deficient host addressed the question whether a systemic, endocrine effect of βB was responsible for the mammary underdevelopment. The transplanted glands were harvested at late pregnancy and normal development was observed (Fig. 6A,C,E). The contralateral endogenous βB-deficient gland had the typical appearance of a mutant gland (Fig. 6B,D,F). In the opposite experiment, the transplantation of $\beta B^{-/-}$ fat pads into wild-type mice, we succeeded only in one case to get limited development of the mammary epithelium (data not shown). This demonstrates that systemic βB is not necessary for mammary development, and it identifies the stroma and/or the epithelium as the sites for the defect in the βB-deficient mice. The second set of transplantation experiments was aimed to identify whether the growth-stimulatory effect of βB was of paracrine nature and mediated by the stroma, as compared to an autocrine or intracrine effect in the epithelial compartment. A small piece of mammary tissue from βB-deficient females was transplanted into cleared fat pads from virgin wild-type mice. In this situation, the mutant mammary epithelium penetrates the host fat pad and becomes associated with wild-type stroma. These experiments allowed us to analyze development of βB-deficient mammary epithelium in a wild-type fat pad in a hormonal environment unaffected by a possible perturbance of pituitary and ovarian hormone levels caused by the absence of the βB subunit. While one fat pad hosted the mutant epithelium the other carried a wild-type transplant. These mice were mated two months after transplantation and mammary tissues were analyzed after the mice had given birth. Both the wild-type (Fig. 7B,D) and the mutant (Fig. 7A,C) transplants grew out to a similar extent. The ducts fully penetrated the fat pad and extensive lobulo-alveolar units had formed. This demonstrates that βB produced by the stroma can rescue the development of mutant mammary epithelium. Clearly, mammary epithelial cells do not require an autocrine, cell autonomous function of the βB subunit.

DISCUSSION

Inactivation of the activin/inhibin *βB* gene results in incomplete mammary development and failure of lactation. Ductal elongation is incomplete, end buds persist throughout pregnancy and morphogenesis of secretory alveoli is reduced. The altered ductal architecture and the persistence of end buds reflect a perturbance in growth regulation, which results from a lack of stimuli from the mammary stroma. Our results demonstrate that stromal-derived activin B, activin AB or inhibin B, or a combination of the three, are obligate for

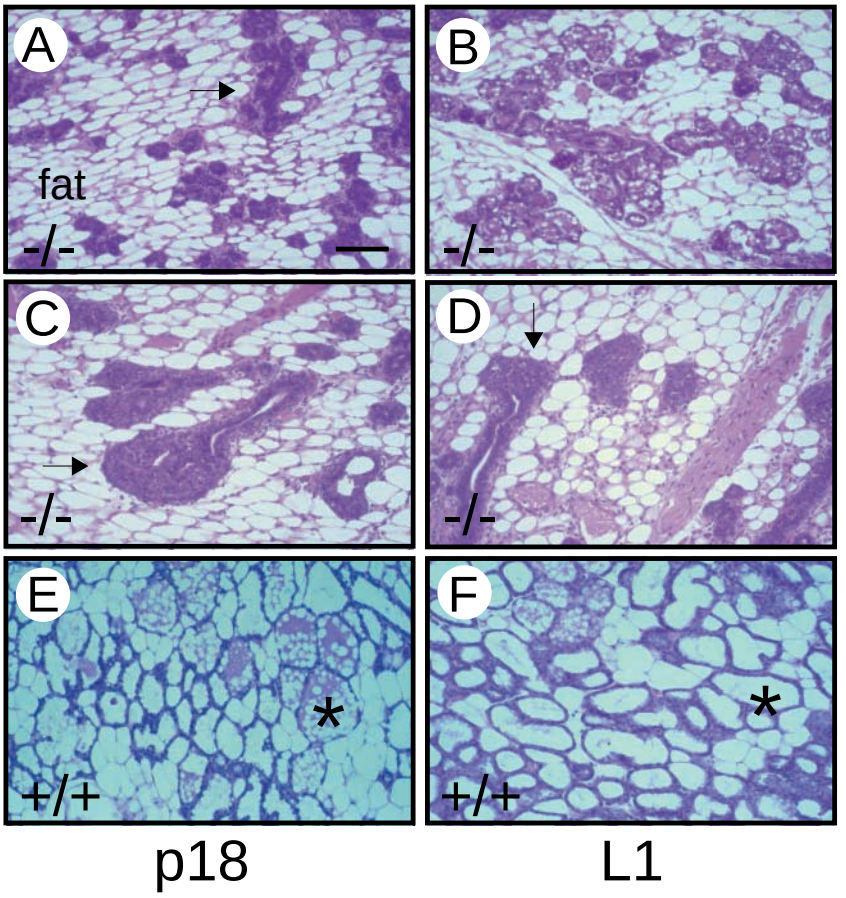

Fig. 3. Alveolar development in $\beta B^{-/-}$ mice. Histological analysis of mammary glands from late pregnant (A,C,E) and postpartum (B,D,F) mice. (A-D) Tissue from $\beta B^{-/-}$ mouse; (E,F) wild-type mouse. (A) Alveoli (arrow) are small and underdeveloped and are embedded in adipose tissue. (B) A slight increase in alveolar size is observed after parturition in $\beta B^{-/-}$ mice. The lumina fail to expand and contain a dense secretion. At day 18 of pregnancy (C) and after parturition (D) endbuds (arrow) persist in $\beta B^{-/-}$ mice. They are large and have disorganized centers. (E) 18 day pregnant wild-type mouse. Alveoli start to expand and secretion containing large lipid droplets accumulates in the lumen (asterix). (F) Tissue from postpartum wild-type mouse. The lumina have expanded further and the luminal contents have been removed by the suckling pups. Bar, 300 μm (A,B); 50 μm (C,D); 150 μm (E,F).

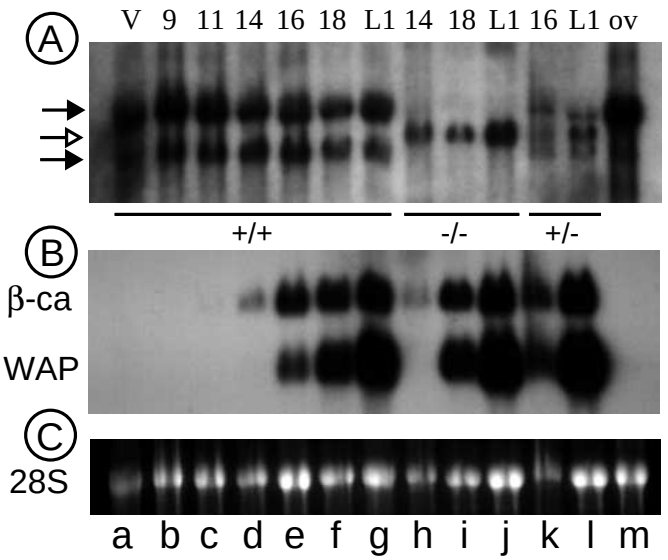

Fig. 4. Expression of βB and milk proteins during mammary development. Mammary gland RNA was isolated from mature virgins (V), at 9, 11, 14, 16 and 18 days of pregnancy and within 1 day after parturition (L1). As a positive control RNA from ovary (ov) was analyzed. (A) Constitutive expression of a 4.5 and 3.5 kb (filled arrows) transcript from the *βB* gene is seen at all stages of mammary development. A 4 kb transcript (open arrow) from the disrupted allele is detected in +/– and –/– tissues. (B) Similar levels of β-casein and WAP are expressed in wild-type (+/+), heterozygous (+/–) and null (–/–) mice. (C) Ethidium bromide staining of 28S ribosomal RNA.

mammary development and that their loss can not be counterbalanced by the closely related activin A and inhibin A.

Ductal morphogenesis

At the time of birth, the mammary anlage consists of a few branching ducts close to the nipple. Rapid outgrowth of the ducts commences with the onset of puberty around 4 weeks of age and is completed between 8 and 10 weeks when the ducts have reached the borders of the fat pad (Daniel and Silberstein, 1987). Terminal end buds (TEB) are specialized structures at the tips of the growing ducts and represent the site of most intensive cell proliferation (Humphreys et al., 1996). These club-shaped TEBs are composed of two types of epithelial cells, the cap cells and the body cells, which can be distinguished by morphology and expression of marker proteins. The cap cells contact the basal lamina. They express P-cadherin and smooth muscle cell markers and are thought to be precursors of the myoepithelial cells that surround lobulo-alveolar units and the ductal system. The body cells are located in the center of the TEBs and are characterized by expression of E-cadherin and specific keratins (Daniel et al., 1995). Ductal morphogenesis and lumen formation in the TEB is accomplished by a highly regulated balance of cell proliferation and cell death (Humphreys et al., 1996). The inability of ducts to elongate and fully penetrate and fill the fat pad in βB-deficient mice could be caused by reduced epithelial cell proliferation in the TEBs through the absence of stromal factors. Alternatively, it is also possible that an imbalance of the different peptides due to the absence of the βB subunit causes the stromal defect. Locally applied growth factors have been shown to affect ductal elongation and branching. TGFα and EGF stimulate ductal growth in ovariectomized mice (Coleman et al., 1988; Snedeker et al., 1991) while they stimulate lobulo-alveolar development in virgin mice stimulated by estrogen and progesterone (Vonderhaar, 1987). TGFβ inhibits ductal outgrowth and causes regression of the TEBs (Silberstein and Daniel, 1987). It has been shown to affect directly the synthesis and deposition of extracellular matrix material and thereby influence branching pattern and growth (Williams and Daniel, 1983; Daniel et al., 1989; Silberstein et al., 1992). The developmentally regulated expression patterns of tachykinins (Weil et al., 1995), members of the fibroblast growth factor (Coleman-Krnacik and Rosen, 1994) and wnt (Gavin and McMahon, 1992; Weber-Hall et al., 1994) families also suggest that they may participate in the control of mammary development.

Alveolar development

Alveolar proliferation is initiated during pregnancy and is completed with lactation when the entire fat pad is filled with lobulo-alveolar units. In βB-deficient mice, pregnancy induced alveolar development is incomplete and the fat pad is sparsely populated. Alveolar outgrowth is controlled by systemic hormones, including glucocorticoids, progesterone and estrogen, prolactin and placental lactogen, and possibly growth hormone and EGF (Topper and Freeman, 1980). Their roles have been shown primarily in culture and it is unclear whether they exert their effects through stromal signals. More recent experiments implicate growth modulators synthesized by the mammary gland itself in glandular development (Cunha and Hom, 1996). There is additional evidence that the extracellular matrix influences mammary gland development and function. Overexpression of tissue proteinases in the alveolar cells of transgenic mice induces precocious alveolar growth and differentiation in virgin glands (Sympson et al., 1994; Witty et al., 1995), while an overexpression of a protease inhibitor delays alveolar development (Alexander et al., 1996).

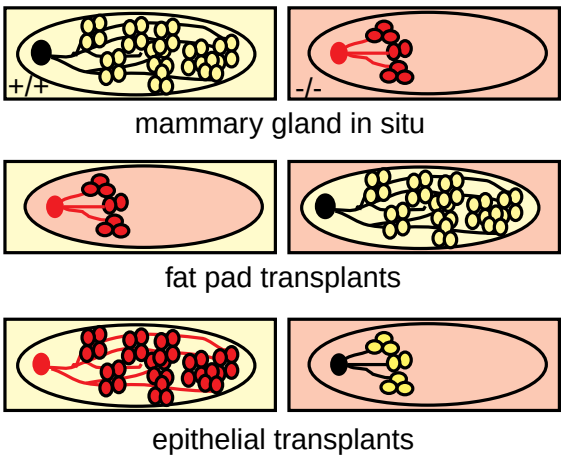

Fig. 5. Schematic presentation of mammary development in transplantation experiments. Either the entire fat pad or mammary epithelium alone was transplanted between mice of $\beta B^{-/-}$ (red) and wild-type (yellow) genotypes. Mammary epithelia, independent of their genotype, develop normally in wild-type fat pads while ductal and alveolar development is inhibited in $\beta B^{-/-}$ fat pads.

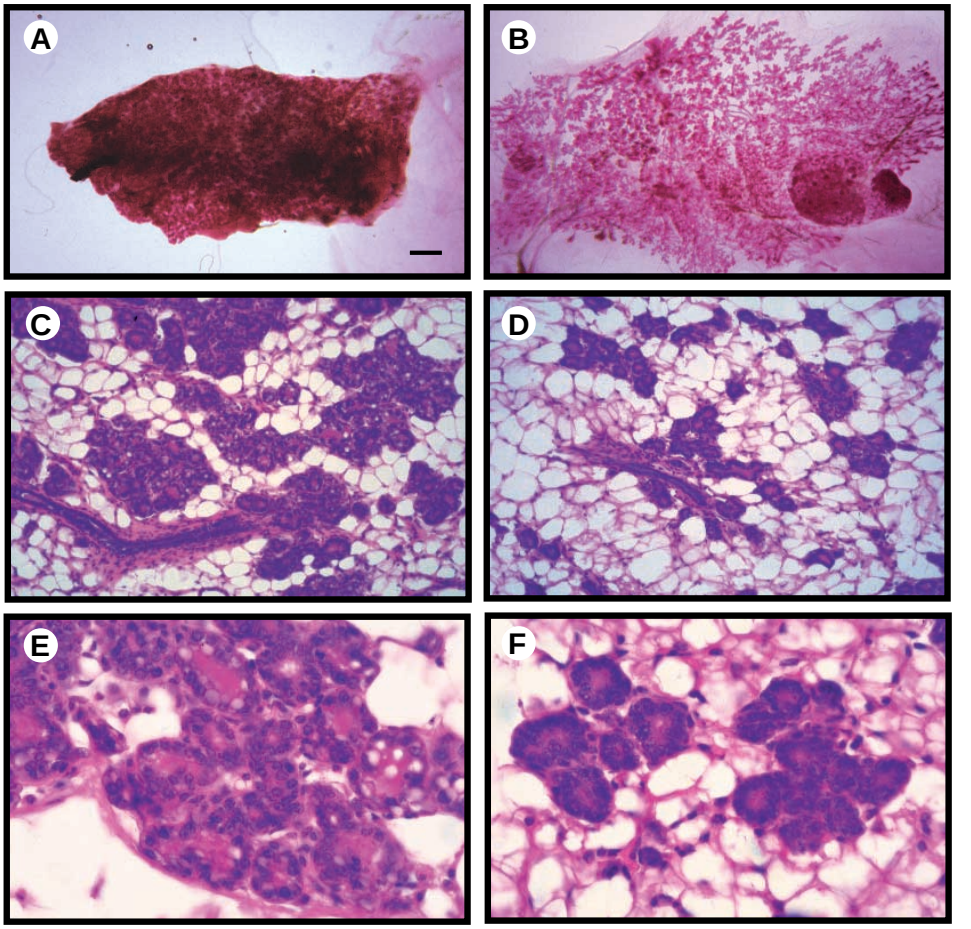

Fig. 6. Wild-type mammary fat pads develop normally in $\beta B^{-/-}$ hosts. Transplanted wild-type fat pad (A,C,E) and endogenous fat pad (B,D,E) harvested at day 15 of pregnancy. (A) Densely filled transplanted fat pad. (B) Sparse alveolar development in contralateral fat pad of $\beta B^{-/-}$ host mouse. (C-F) The difference in alveolar development is more clearly seen in histological sections. Early signs of secretory differentiation are seen in the alveoli of the transplant (C,D) while the alveoli are small and underdeveloped in the –/– gland of the host (E,F). In two out of two experiments, good development of the transplanted fat pad was observed. Of two $\beta B^{-/-}$ fat pads transplanted into wild-type mice, poor epithelial growth was found in one. Bar, 1 mm (A,B); 300 μm (C,D); 50 μm (E,F).

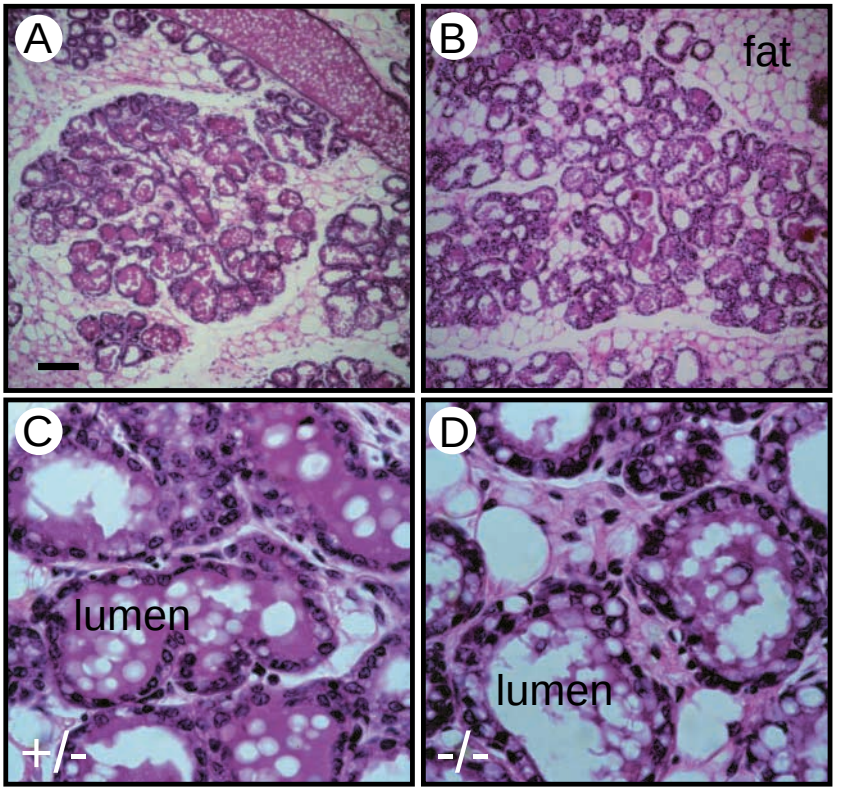

Fig. 7. Development of epithelial transplants in wild-type fat pads. On day 15 of pregnancy, no difference in alveolar development is seen between +/– (A,C) and –/– (B,D) epithelial transplants developing in a wild-type host. Three out of five –/– epithelia showed complete development when transplanted into a wild-type fat pad. In the inverse experiment, one out of five wild-type epithelial transplants into $\beta B^{-/-}$ stroma recovered contained poorly outgrown ducts. Bar, 300 μm (A,B) and 50 μm (C,D).

A connection between local growth factors and hormonal signals has been demonstrated in the uterus where TGFβ mediates the progesterone suppression of an epithelial metalloprotease by the adjacent stroma (Bruner et al., 1995).

A participation of locally produced inhibin in alveolar development has been implied by the observation that treatment of rats with chorionic gonadotropin induces alveolar development (Russo and Russo, 1994), which is accompanied by an increase and shift of immunoreactivity for inhibin from the stroma to the alveolar cells (Alvarado et al., 1993). In addition, mammary epithelial cell lines have been shown to contain activin receptors I, II and IIB, and thus have the potential to respond to activin signals (Ying and Zhang, 1996; Ying et al., 1995). The growth of primary and transformed mammary epithelia, and tubule formation by human mammary organoids in response to hepatocyte growth factor/scatter factor (HGF/SF) is inhibited by activin A (Liu et al., 1996).

Stromal dependence of mammary development

Mammary gland development is dependent on epithelial-stromal interactions. From its initiation as a small epithelial bud in the embryo, reciprocal and sequential tissue interactions are required for mammary morphogenesis (Kratochwil, 1975; Sakakura, 1987; Cunha, 1994). The expression patterns of many growth factors in mammary development suggest their involvement in these processes (Cunha and Hom, 1996). LEF-1 (van Genderen et al., 1994), HGF/SC (Yang et al., 1995; Niranjan et al., 1995; Soriano et al., 1995), keratinocyte growth factor (Ulich et al., 1994) and neuregulin (Yang et al., 1995) have been identified as factors that are produced in the mesenchyme and thus may mediate short-range signals to influence mammary epithelial growth. All of these molecules are also required for the development of other organs and their inactivation does not show the same exclusive effect on mammary glands as does the βB subunit.

Our transplantation studies localize the defects of ductal growth and alveolar differentiation in βB-deficient mice to the mammary stroma. There is no requirement for systemic βB since wild-type fat pads can support full epithelial development in βB-deficient hosts. Moreover, epithelium-derived βB is not required as βB-deficient epithelia develop to the same extent in wild-type fat pads as control epithelia. A paracrine action of activins and inhibins is further supported by the observation that the organs affected in mice with a βA mutation, namely development of the secondary palates, teeth and whiskers (Matzuk et al., 1995a), all depend on epithelial-mesenchymal interactions. Furthermore, branching morphogenesis of embryonic salivary gland, kidney and pancreas in organ culture can be reversibly disrupted by activin A while no effect was seen with inhibin A (Ritvos et al., 1995). In testicular development, inhibin appears to have paracrine function while activin has an autocrine effect on Sertoli cells (Moore et al., 1994). It is still unclear whether the effective growth factor that is missing in the βB-deficient gland is activin B, AB, inhibin B or all of these. Pure preparations of these factors will be required to dissect the mechanisms of their actions on the different cell populations of the mammary gland and help understand the unique function of these multipotent and widely expressed growth factors in the mammary gland.

Genes and mammary development

Disruption of mammogenesis and lactogenesis has been observed in mice from which different genes had been deleted by homologous recombination. In contrast to the βB-deficient gland described in this study and the estrogen receptor knockout mice (Lubahn et al., 1993), ductal outgrowth during puberty was not impaired by the absence of cyclin D1 (Sicinski et al., 1995), the progesterone (Lydon et al., 1995) and prolactin (Ormandy et al., 1997) receptors and the *Stat5a* gene (Liu et al., 1997). The absence of these genes affected only lobulo-alveolar development. The difference in phenotype could be explained by the different nature of the molecules. While inhibins and activins are growth factors that signal mammary development through the stroma, cyclin D1, *Stat5a*, and the progesterone and prolactin receptors by definition exert their function only in the cell in which they are expressed.

In this study, we have shown that locally produced βB subunit is required for ductal and alveolar mammary growth. Although the potential of mesenchymal signalling on the epithelial compartment has been shown in organ explants, implant and reconstitution experiments, the βB-deficient mice provide the first genetic evidence for stromal signalling in the adult mammary gland in vivo.

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